Chapter Six

Summary

If people do not believe that mathematics is simple, it is only because they do not realize how complicated life is.

— John Louis von Neumann (1903–1957)

Advances in fluorescent probing and microscopic imaging technology have revolutionized biology in the past decade and have opened the door for studying subcellular dynamical processes. A simple description of sometimes complex patterns of movement in living cells may give insight in the underlying mechanisms governing these movements. However, accurate and reproducible methods for processing and analyzing the images acquired for such studies are still lacking. Since manual image analysis is time consuming, potentially inaccurate, and poorly reproducible, many biologically highly relevant questions are either left unaddressed, or are answered with great uncertainty. Hence, the development of automated image analysis techniques for accurate and reproducible tracking and motion analysis of subcellular structures from time-lapse microscopy image data is crucial.

Recent results in psychophysics and human vision research have revealed the highly integrated nature of vision systems in using spatial, temporal, and prior information [23]. Local motion signals are often ambiguous, and many important motion phenomena can be explained by hypothesizing that the human visual system uses temporal coherence to resolve ambiguous inputs. It has therefore been proposed that input data are temporally grouped and used to predict and estimate the motion flows in image sequences. Such temporal grouping can be expressed in terms of a Bayesian generalization of standard Kalman filtering. Existing tracking techniques, whether commercial or academic, generally make very limited use of temporal information and prior knowledge.

The subject of this thesis is particle filtering methods and their application for multiple object tracking in different biological imaging applications. Particle filtering (PF) is a technique for implementing recursive Bayesian filtering by Monte Carlo sampling. A fundamental concept behind the Bayesian approach for performing inference is the possibility to encode the information about the imaging system, possible noise sources, and the system dynamics in terms of probability densities. Nevertheless, the Bayesian tracking framework is rather a "recipe" than a ready-to-use solution to a given problem, which should be implemented in practice, for example using the

PF approximation. In general, the construction of particle filters is not unique, and for any given application will lead to different algorithmic trade-offs. As stated by some methodologists in this field "the design of efficient particle filters is still more of an art than a science" [126]. In this thesis, a set of novel PF based methods for subcellular motion analysis is developed. The applicability of these methods for robust and accurate detection and tracking of large numbers of small objects in 2D and 3D image sequences obtained by fluorescence microscopy imaging as well as for dynamics analysis using kymoghraphs is demonstrated and evaluated.

Contrary to the conventional two-step (detection and linking) approaches to tracking, Bayesian tracking does not require a separate object detection procedure. Nevertheless, robust and accurate detectors can be efficiently used in the Bayesian framework, especially to initiate new tracks and terminate existing ones, when the object appears in or disappears form the field of view, respectively. In Chapter 2, the performance of six unsupervised and two supervised (machine learning) detection methods for the detection of small spots in fluorescence microscopy images is quantitatively evaluated. It is shown that overall, the supervised methods (AdaBoost and Fisher discriminant analysis) perform better, in that they show the highest true positive rate (at very low false positive rate) and the lowest sensitivity to parameter changes, for all types of image data considered. Nevertheless, the differences in performance are not large compared to some of the unsupervised methods, especially the so-called h-dome detector (HD) proposed in the chapter. Based on extensive experiments, the conclusion is drawn that when a detector with overall good performance is needed, the mentioned supervised detectors or the unsupervised HD detector are to be preferred. The disadvantage of the supervised methods is that they rely on a training stage, which involves the extraction of positive and negative samples from the image data beforehand. This requires manual annotation of thousands of objects in order to achieve sufficient discriminating power, and is extremely tedious, time consuming, and observer dependent. Taking this into account, the unsupervised HD detector is much easier to use in practice. Finally, when the SNR is sufficiently high (> 5 as a rule of thumb), the other unsupervised detectors perform just as well, and require only minimal adjustment of their parameters to the specific application.

Chapter 3 describes the derivation of a novel particle filter for quantitative analysis of subcellular dynamics, in this case for microtubule growth analysis. The algorithm exploits prior information about the microtubule dynamics and imaging process, which makes it perform superior in the presence of severe noise in comparison with existing frame-by-frame approaches, which break down at SNR < 4–5 [26, 32]. Additionally, the algorithm naturally deals with photobleaching effects. Experiments on synthetic data confirm that the proposed PF yields reliable tracking results even in data with SNR as low as 2, contrary to two other popular tracking tools, and that it is potentially more accurate than manual tracking by expert human observers. Applied to real fluorescence microscopy image sequences from microtubule dynamics, the algorithm performs comparable to human observers. This is explained by the fact that the latter experiments were limited to comparing distributions and averages, which may conceal small local discrepancies, especially when the objects' velocities vary over time. Instant velocities were also analyzed per track but could not be quantitatively validated due to the lack of ground truth.

Since common Bayesian tracking algorithms are designed to deal with only one specific type of motion, they may fail when used for biological applications where more complex motion patterns need to be analyzed. Therefore, in Chapter 4, the algorithm developed in Chapter 3 is extended to be capable of tracking different types of subcellular objects with different types of motion patterns. The tracking accuracy is improved by using marginalization of the filtering distribution and one of the state variables, for which the optimal solution (the Kalman filter) is used. In addition, improved robustness is achieved by integrating a jump Markov system into the framework, which allows the use of multiple dynamics models for object motion prediction. The proposed algorithm is tested on synthetic image data as well as on real time-lapse fluorescence microscopy data acquired for studying the dynamics of three different types of intracellular objects: microtubules, vesicles, and androgen receptors. Results from synthetic data experiments clearly show the superiority of the proposed algorithm over manual tracking as well as previous Bayesian tracking approaches, which were already demonstrated to be superior to alternative non-Bayesian tracking algorithms. The real-data experiments confirm the validity of the tracking results produced by the proposed algorithm. Based on these results, the algorithms are now being explored in practice for addressing specific biological questions.

Finally, Chapter 5 demonstrates the applicability of PF methods to another biological application: the analysis of microtubule dynamics *in vitro*, imaged using differential interference contrast microscopy. A novel algorithm is proposed that combines variable-rate particle filtering (VRPF) and multiscale trend analysis for analyzing the motion of the growing or shrinking tips of microtubules in 2D spatiotemporal images (kymographs). The proposed VRPF optimally combines image information and prior knowledge about the underlying microtubule dynamics and is capable of following the microtubule end even in situations where rapid motion changes (after rescue or catastrophe) occur. As demonstrated by experiments on synthetic data, the method is capable of accurate estimation of the important kinematic parameters. In these experiments, the error of locating the microtubule tip in kymographs is measured and its influence on the estimation of the important kinematic parameters (growth and shrinkage velocities, rescue and catastrophe frequencies) is studied. From theoretical considerations it is known that even relatively small errors in tip localization can lead to large errors in the final parameter estimates, due to the nonlinear relationship between the estimated slopes and the computed velocities. Indeed, the experimental results show increased uncertainty in velocity estimation for higher velocities. Applied to real data, the proposed method produces parameter estimates in accordance with estimates obtained manually by expert biologists.